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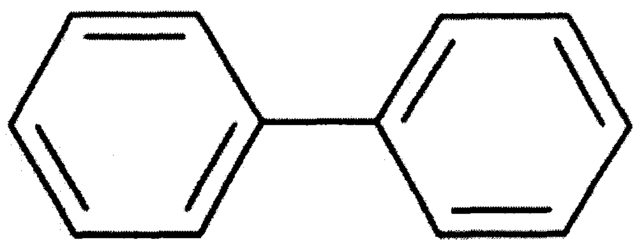
Biphenyl

CAS Number 92-52-4



U.S. EPA HPV Challenge Program Submission

Submitted by:

SOCMA Biphenyl Working Group

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Executive Overview

1,1'-Biphenyl, CAS no. 92-53-4, is an aromatic hydrocarbon that is naturally occurring and is a common combustion product. It is commercially synthesized from benzene or xylene. It is a sublimeable white to yellow crystalline solid with a unique and characteristic odor and a melting point of 70” C. It has low volatility (boiling point 254°C and vapor pressure of 0.0119 hPa @ 25°C) and is relatively insoluble in water (water solubility 7.88 mg/L). Its most extensive use is as a chemical intermediate but it is also used as a heat transfer fluid.

In the environment, based on physicochemical and experimental data, Biphenyl has potential to bioaccumulate (Log $K_{o/w}$ = 4.01) and will distribute primarily to soil and water where it will be subject to limited volatilization and rapid biodegradation under conditions favorable to bacteria. It is stable to hydrolysis but expected to react rapidly with atmospheric hydroxyl radicals with a half-life of about 18 hours. Biphenyl is toxic to aquatic species, with an acute LC_{50} for freshwater fish in the range of 1 to 2 mg/L and daphnia of 0.3 to 1 mg/L; growth inhibition of green alga has also been demonstrated in the range of l-5 mg/L. The potential for bioaccumulation and adverse effects on aquatic species is offset by its facile biodegradation in the environment.

The acute oral toxicity of Biphenyl is low with an LD_{50} value of 2400 mg/kg being typically reported for rat gavage studies. Exposure of rats to saturated vapor for 8 hours did not produce any significant adverse effects and the dermal LD_{50} in rabbits is greater than 2000 mg/kg.

A large number of repeated-dose, subchronic and chronic studies in several species illustrate that Biphenyl is well tolerated orally at lower exposure levels. Repeated dosing, however, at high levels can result in adverse effect to the kidney and bladder with urinary calculus formation. Other than the urinary system, no systemic target organs have been identified. Repeated inhalation of vapor at 50 ppm by rats was found to result in hyperplasia of the trachea; however, exposure to 25 ppm produced only minimal effects.

Adequate *in vitro* tests of genetic toxicity for Biphenyl are available. Multiple *Salmonella typhimurium* reverse mutation assays show lack of mutagenic activity in the presence or absence of metabolic activation and *in vitro* DNA damage studies produce primarily negative results; however, some tests have been positive. The overall preponderance of data suggests that Biphenyl is not genotoxic. .

Developmental toxicity has been investigated using an OECD 4 14 Guideline-like study in mice and an older, but adequate study in rats. These investigations, both conducted by oral gavage at 0, 100, 250, 500 or 1000 mg/kg-day, indicate that Biphenyl affects the conceptus only at maternally toxic doses, and even at those levels no major malformations occurred. The maternal and developmental NOAEL was found to be 500 mg/kg-day.

The combination of these modem negative developmental toxicity studies with findings from subchronic studies showing lack of effect on reproductive organs fulfills the current requirement for reproductive toxicity information. In addition, there is a three-generation reproduction study and a limited one-generation reproductive investigation in rats that have been reported showing lack of specific reproductive toxicity.

It is concluded that the available information adequately fills all the data elements of the HPV Program for Biphenyl with the exception of in vivo mutagenicity. Thus an in vivo mutagenicity study on biphenyl will be conducted.

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Testing Plan and Rationale

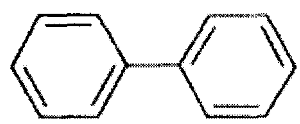
Testing Plan in Tabular Format

CAS Number 92-52-4 1,1'-Biphenyl	Information Available?	OECD Study?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?
HPV Endpoint							
Physical Chemical							
Melting Point	Y	N	N	Y	N	Y	N
Boiling Point	Y	N	N	Y	N	Y	N
Vapor Pressure	Y	N	N	Y	N	Y	N
Partition Coefficient	Y	N	N	Y	N	Y	N
Water Solubility	Y	N	N	Y	N	Y	N
Environmental & Fate							
Photo-Degradation	Y	N	N	N	Y	Y	N
Water Stability	Y	N	N	Y	N	Y	N
Transport	Y	N	N	N	Y	Y	N
Biodegradation	Y	Y	Y	Y	N	Y	N
Ecotoxicity							
Acute Fish	Y	N	Y	Y	N	Y	N
Acute Invertebrate	Y	Y	Y	Y	N	Y	N
Acute Algae	Y	N	N	Y	N	Y	N
Toxicity							
Acute	Y	N	N	Y	N	Y	N
Repeated Dose	Y	N	Y	Y	N	Y	N
Genetic Toxicology "in vitro"	Y	N	Y	Y	N	Y	N
Genetic Toxicology "in vivo"	Y	N	Y	Y	N	N	Y
Reproductive	Y	N	N	Y	N	Y	N
Developmental	Y	Y	Y	N	N	Y	N

Introduction

Biphenyl, CAS no 92-52-4, is an aromatic hydrocarbon that is a colorless solid at room temperature and has what is described as a pleasant peculiar odor (1). It is used as an intermediate in the production of a variety of compounds such as: emulsifiers, optical brighteners, crop protection products and plastics, as a dyestuff carrier in textiles and copying paper and as a heat transfer fluid. Biphenyl also occurs naturally in coal tar, crude oil and natural gas (2).

Its structure is shown below:



1,1'-Biphenyl

Biphenyl is also known as (2):

- Bibenzene
- 1,1'-Biphenyl
- Diphenyl
-
- Lemonene
- Phenylbenzene

Exposure in industrial applications is limited by process controls, protective equipment, a very low vapor pressure and excellent warning properties due to its characteristic odor. The ACGIH TLV for Biphenyl is 0.2 ppm.

A broad spectrum of physicochemical, fate and toxicity studies have been conducted on Biphenyl. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are tilled by high-reliability studies on Biphenyl. Where direct data are not available or data are sparse, surrogates or estimation methods are used to fill the data element. This activity is encouraged by the U.S. EPA and other regulatory authorities to avoid unnecessary testing and animal usage.

Physicochemical Data

Physicochemical data for Biphenyl are available from the literature.

Table I: Physicochemical Properties of Biphenyl	
Melting Point	69-71” C (1)
Boiling Point	254-255” C @ 1010 hPa (1)
Vapor Pressure	0.0119 hPa @ 25°C (3)
Partition Coefficient	Log $K_{o/w}$ = 4.01 (4)
Water Solubility	**7.28 mg/L** @ 25” C (5)

These properties indicate that below 70” C, Biphenyl is a volatile solid with low to limited water solubility. The value of the partition coefficient suggests that Biphenyl will partition preferentially into fat; therefore, on the basis of only the octanol-water partition coefficient, Biphenyl is considered to have potential for bioaccumulation; however, if biodegradation and oxidative metabolism are taken into consideration, actual bioaccumulation is much less. The International Program on Chemical Safety (IPCS) has concluded, “. . . bioaccumulation of the chemical should be of minor importance for aquatic organisms” (6).

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Multiple screening studies using activated sludge as the inoculum have been conducted to assess the biodegradability of Biphenyl. These studies indicate that Biphenyl can be considered readily biodegradable. Biphenyl was tested at 100 mg/L in the MITI test and achieved 66% of the theoretical BOD after two weeks (7). At an initial concentration of 0.8 mg/L, Biphenyl reportedly achieved 100% of the theoretical oxygen uptake in an OECD 301 D test (8). In a river die-away study (presented in the robust summaries), Biphenyl at concentrations up to 100 mg/L was shown to undergo almost complete mineralization within a period of eight days without a lag phase (9). Supporting studies showing biodegradability in mixed cultures and with various specific organisms support the ease of Biphenyl’s biodegradation (10). It is speculated that the ease with which natural bacteria degrade Biphenyl without a lag period may be related to Biphenyl’s natural occurrence in the environment and to its occurrence as a common combustion product.

Biphenyl’s photodegradation was estimated using version 1.90 of the Atmospheric Oxidation

Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 6.8 E-12 cm^3/molecule-set; however, the SRC database for hydroxyl radical rate constants (built into AOPWIN) contained an experimental value determined by Atkinson of 7.2 E-12 cm^3/molecule-set, which is essentially identical with the calculated value. Using the default atmospheric hydroxyl radical concentration in APOWIN and the experimentally determined rate constant for reaction of Biphenyl with hydroxyl radical, the estimated half-life of Biphenyl vapor in air is approximately 18 hours (see accompanying robust summary for full details).

Water stability has not been quantitatively determined for Biphenyl. Quantitative stability determinations (e.g. OECD 111) are considered unnecessary for compounds containing only non-hydrolysable groups. Under these conditions the SIDS manual states that consideration should be given to using an estimation method. There is no evidence available in the literature that Biphenyl is unstable in water and the structure is that of a simple aromatic hydrocarbon, which is a class of molecule considered to be water unreactive at environmental pH values. The half-life in water is thus estimated as greater than one year. This estimate is confirmed by the review of Harris, who notes specifically that biphenyls as a class are non-hydrolysable (11).

Volatilization and sorption are important in the transport of Biphenyl in aquatic systems. The Henry's Law constant for Biphenyl(2.5×10^{-4} atm-m^3/mol) suggests that the molecule may undergo volatilization from aqueous solution. A volatilization half-life of 4.3 hours was estimated for Biphenyl in a stream 1 m deep, flowing 1 m/second, with an air current of 3 meters/second (12).

Theoretical Distribution (Fugacity) of Biphenyl in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05. This estimate used the measured vapor pressure of 0.0089 mm Hg, the measured log $K_{o/w}$ of 4.0 1, an experimentally determined Henry's Law constant, and a measured value for the melting point (13). The results for distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.0042 and equal initial distribution to air, water and soil are:

- Air 5.5 %
- Water 28.8 %
- Soil 63.8 %
- Sediment 1.9 %

Recommendation: No additional fate studies are recommended. The available data fill the HPV required elements.

Ecotoxicity

A recent GLP guideline-like study of acute fish toxicity using measured concentrations and flow-though conditions with rainbow trout resulted in an LC_{50} **(192-hour)** determination of 1.36 mg/L with a 95% confidence interval of 0.81-l (14). This finding is in accord with older static tests of Biphenyl on freshwater fish, where 96-h LC_{50} values from 1.5-4.7 mg/L have been reported, with rainbow trout being the most sensitive species (15). In addition to acute studies, an **87-day** early-life-stage study of rainbow trout has been conducted (14). In this study of hatching, development and growth, the NOEC was reported to be 0.229 mg/L Biphenyl and the Maximum-Allowable Toxicant Concentration (MATC) was assigned as 0.275 mg/L.

Daphnia acute studies run under static conditions have produced a relatively narrow range of toxicity values from an EC50 of 0.73 mg/L in one test (16) to 4.7 mg/L in another (17) with *Duphnia magna.* The lowest EC_{50} that has been reported was from a closed flow-through system, where an EC_{50} of 0.36 mg/L and a NOEC of 0.04 mg/L were reported (18). This acute flow through test was used as a range-finding study for setting Biphenyl concentrations in a reproduction test with *Duphnia magna* in the same closed continuous-flow system. The NOEC after 2 1 days of incubation including reproductive function was 0.17 mg/L; the MATC was calculated from this study to be 0.23 mg/L.

The 96 hr growth rate EC50 value in an algae growth inhibition study for a 75%/25% diphenyl oxide/biphenyl mixture and a 100% diphenyl oxide were 1.8 and 2.5 mg/L, respectively (19; 20). Although pure biphenyl was not studied, the lack of a significant difference between the two samples suggests that the response for biphenyl and diphenyl oxide is similar. The measured algal growth 96-hr EC50 value for 75%/25% diphenyl oxide/biphenyl mixture is consistent with the ECOSAR-predicted value for 96 hours of 1.3 mg/L (see Table 2). An algal growth inhibition study on Biphenyl has been conducted by Hutchinson et al. (2 1) using two species of green algae Chlamydomonas angulosa and Chlorella vulgaris that gave 3-hr EC50 values of 1.3 and 3.9 mg/L, respectively. This result is also supported by a growth inhibition study of the green alga Chlorella autotrophica, which was slightly inhibited (4 mm zone of inhibition) at 1 .O mg Biphenyl/plate and totally inhibited (36 mm zone of inhibition) at 10 mg/plate (22).

Table 2: Comparative Aquatic Toxicity of Biphenyl		
	Reported Values	ECOSAR Prediction
Fish, 96-hour static LC_{50} (Rainbow Trout)	1.5 mg/L (15)	1.5 mg/L*
Fish, 192-hour flow through LC_{50} (Rainbow Trout)	1.36 mg/L (14)	
Daphnia, 48 hour flow trough EC50	0.36 mg/L (23)	1.8 mg/L*
Algae, 96-hour EC50 (*Selenastrum capricornutum)*	1.8 mg/L 25% biphenyl/75% diuhenvl oxide (19)	1.3 mg/L*

* Estimated using ECOSAR (22)

Recommendation: The fish, invertebrate, and algal growth inhibition test results are adequate. As ECOSAR-based estimates of toxicity result in an excellent correspondence with the measured values for fish, daphnia, and algae, the overall ecotoxicity data are considered adequate for the purpose of the HPV program.

Metabolism

Facile metabolic conversions of Biphenyl to more polar structures are considered the primary reason why this material does not bioaccumulate to any large extent. The initial metabolites appear to be the same in bacteria as in mammals and these pathways are probably also conserved in fish and invertebrates. In mammals, it has been established that the most prevalent initial metabolite is 4-hydroxybiphenyl. Meyer et al. (25) studied the metabolism of Biphenyl in the rat and reported the primary urinary metabolites as 4-Hydroxybiphenyl (7.7% of dose) and 4,4'-Dihydroxybipehnyl (11.4% of dose). The total urinary recovery 96 hours after administration was 29.5% of the dose and the metabolites detected were conjugates of the mono-, di-, and trihydroxy derivitives of Biphenyl as well as the meta- and para-methyl ethers of the catecholic compounds. These researchers also demonstrated that Biphenyl must be hydroxylated and conjugated prior to biliary excretion and found 5.2% of the dose in the 24 hr bile as conjugates, mainly of 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl, and 3,4,4'-trihydroxybiphenyl. Other previously undetected minor metabolic products that were identified in the rat were: 3,4'-dihydroxybiphenyl, 3,4,4'-trihydroxybiphenyl, 3,4'-dihydroxy-4-methoxybiphenyl and 4,4'-dihydroxy-3-methoxybiphenyl

Urinary calculi are a consistent finding in repeated dose studies of Biphenyl at high dose levels, with males showing a much higher incidence than females-(26). A recent study demonstrated that there is a sexual dimorphism in the composition of the urinary calculi with the male's calculi being composed primarily of potassium 4-hydroxybiphenyl-o-sulfate whereas the calculi in female rats are composed mainly of 4-hydroxybiphenyl and $KHSO_4$ (27). Moreover, the calculi have different physical properties and appearance. Photomicrographs and the results of FT-IR analysis indicated that the calculi in males have a multilayer structure consisting of alternating layers of potassium 4-hydroxybiphenyl-o-sulfate and calcium phosphate. In contrast, the calculi in females do not have a multilayer structure, but have open holes in which needle-shaped crystals are sometimes present. This could account for much of the difference in sensitivity between male and female rats.

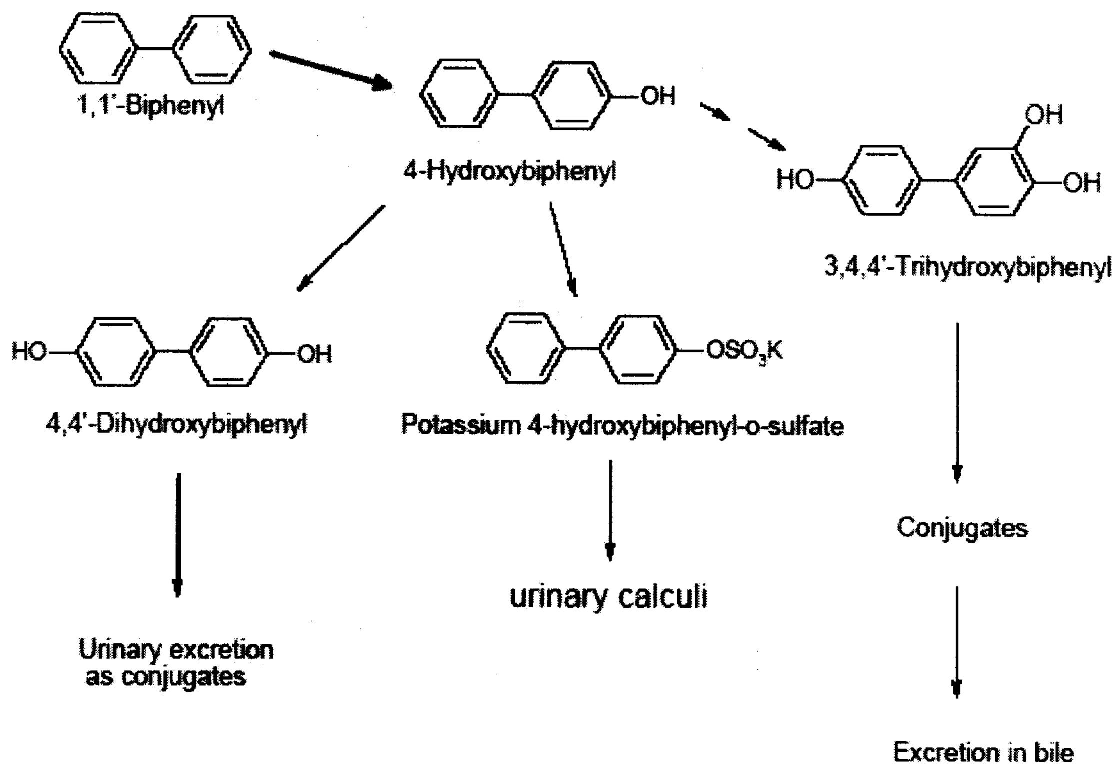


Figure 1: Principle Metabolic Routes of Biphenyl in the Rat

Figure I: Principle Metabolic Routes of Biphenyl in the Rat

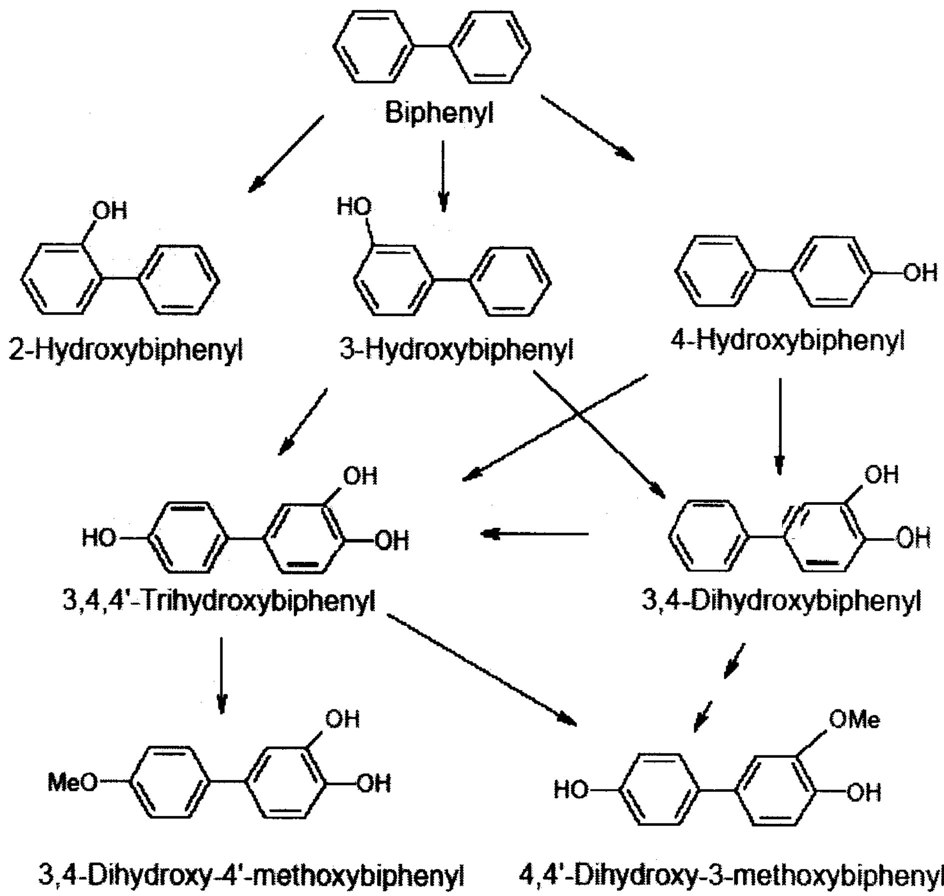


Figure 2: Some of the Known Biphenyl Metabolites

Figure 2: Some known Biphenyl Metabolites

Health Effects

Acute Toxicity

Oral Exposure

Multiple determinations of the oral LD_{50} of Biphenyl reported with LD_{50} values ranging from 2 180 to 5040 **mg/kg** indicating a low order of acute oral toxicity for this material. Robust summaries have been prepared from the 1976 and the 1947 studies listed below. The 1949 study gave a lower LD_{50} but the material was listed as "purity unknown". A later test by the same laboratory (Mellon Institute) in 196 1 using a material described as refined and approximately 99% pure produced a somewhat higher LD_{50}. Overall, the results fall into a reasonably consistent range considering different strains of rats were used with different vehicles and varying purities of test material.

Oral LD_{50}	Year	Sex studied	Comment	Reference
2180	1949	Male	Purity unknown	28
2400	1976	M & F		29
3280	1947	Not reported		30
3730	1961	Male	Refined material	31
4500	1988			32
5040	1975			33

Table 3. Acute Oral Toxicity of Biphenyl

Inhalation Exposure

No deaths were observed when a group of six female rats were exposed to saturated vapor and mists of purified Biphenyl for 8 hours (3 1). The actual concentration was not measured but based on the vapor pressure at 20°C and 100" C (5.5 hPa in ECB IUCLID 2000). The vapor concentration is calculated to be in the area of 100 ppm and the aerosol concentration (from condensation of supersaturated vapors) could have been in the range of 20-50 mg/L.

Dermal Exposure

A limited study has indicated that the dermal LD_{50} of Biphenyl applied to rabbits as a 40% solution/suspension in corn oil, is greater than 5010 mg/kg-bw (29).

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all requirements of current OECD guidelines in all cases, the weight of evidence shows the oral and dermal toxicity is very low. Likewise, the limited study of acute saturated vapor by inhalation provides important and scientifically defensible information about vapor toxicity. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Multiple repeated dose (14 day through chronic) studies have been conducted with Biphenyl. For the purposes of the HPV program, four have been selected for presentation and summarization. The first is the chronic feeding study by Ambrose (34). The second and third are the 2-year feeding studies in rats and mice conducted by the Japan Bioassay Research Center (26; 35; 36). The final study is a 13-week vapor inhalation study in mice conducted by Cannon Laboratories (37). These were selected because of their duration, relevance of the route of administration, and because they cover two species for carcinogenicity. The Ambrose study is identified as the critical repeated-dose study for the HPV program because of the long duration, the use of several dose levels and the scope of the study (which included two satellite tests of reproductive function).

Oral Exposure

In this chronic feeding study reported by Ambrose et al. (34), 15 rats of each sex were fed diet containing 0, 10, 50, 100,500, 1000, 5000 or 10000 ppm (0.001 to 1% w/w, ca 0.75, 3.75, 7.5, 37.5, 75,375 or 750 mg/kg-day). At 5000 ppm, increased liver and kidney weights were observed in females. Concentrations of 5000 and 10000 ppm resulted in shortened lifespan, growth inhibition and lowered hemoglobin values (growth inhibition and reduced hemoglobin levels were attributed to decreased food intake). Treatment related histopathological changes in the kidneys were observed at 5000 ppm and above. The NOAEL was considered 1000 ppm (ca 75 mg/kg-day).

A chronic study using F344/DuCrj rats, performed by the Japan Bioassay Research Center, according to standard protocols, showed a significant increase in neoplastic and non-neoplastic lesions of the urinary bladder and calculi within the urinary bladder of high dose males (26; 35). High dose females had a significant increase in non-neoplastic lesions of the urinary bladder and a much lower incidence of urinary bladder calculi. In this 104-week study, dietary concentrations of Biphenyl were 0,500, 1500, or 4500 ppm (0, 38, 113, or 338 mg/kg body weight per day).

In this study, a dose-dependent increase in hyperplasia of the renal pelvis epithelium was reported. Histopathological findings for the kidneys and urinary bladder are summarized in the companion Robust Summary. Other findings included increased serum levels of alkaline phosphatase, aspartate transaminase, and alanine transaminase and an increased urea nitrogen level in low-dose males and mid-dose females, which became more pronounced with increasing doses. Hematological effects were reported in mid- and high-dose females and in high-dose males. A LOEL of 38 mg/kg, based on the clinical chemistry results, was derived from these data according to the CICAD . The anemia in male rats was considered to be due to hematuria (personal communication-Y. Umeda). The anemia in female rats was only observed in old animals in the chronic bioassay and was not observed at dose levels as high as 16,000 ppm in the 13 week study.

A companion chronic study using Crj:BDF1 mice was conducted by the Japan Bioassay Research Center (35; 36). In this study, groups of 50 mice of each sex were given diets containing 0, 667, 2000, or 6000 ppm Biphenyl (0, 100,300, or 900 mg/kg body weight per day) for 104 weeks prior to sacrifice and complete histopathologic examination. An increase in liver tumors (hepatocellular adenomas and carcinomas) was observed in the females at doses of 300 and 900 mg/kg body weight per day. In addition basophilic cell foci were observed at an increased incidence in low dose males and middle and high dose females. The increase observed in low dose males did not follow a typical dose response curve and is not believed to be dose-related. The increase observed in middle and high dose female mice also did not follow a typical dose response curve but is believed to be treatment-related. According to the CICAD, degenerative changes of the nasal cavity respiratory epithelium were reported in male and female mice at doses $\geq$ 100 mg/kg body weight per day and degenerative changes of the respiratory nasopharynx at doses ≥300 mg/kg body weight per day. Other findings included variations in serum enzyme levels (increase in alkaline phosphatase, aspartate transaminase, and alanine transaminase). Urea nitrogen level in the males and females ingesting biphenyl were increased in a dose-related manner but effects observed in the high dose group were still minimal. In female mice receiving ≥300 mg Biphenyl/ kg body weight per day and in the high-dose males, degenerative changes in the kidney (increased mineralization of the inner stripe of the outer medulla, increase in desquamation of the epithelium of the renal pelvis) were also observed. Body weights of high-dose animals and middle dose females were reduced from control values, however food consumption was decreased only slightly or comparable to control values.

Several studies have been conducted by the Japan Bioassay Research Center to help explain the increased incidence of hepatocellular adenomas and carcinomas in female mice (39; 40 and personal communication – Y. Umeda). These studies help to show the increased incidence of hepatocellular tumors may be due to peroxisome proliferation. The relative liver weights in female mice were increased 9 and 23% following 13 weeks administration of biphenyl at 4000 and 8000 ppm, respectively (personal communication- Y. Umeda). Electron microscopic examination of a high dose female mouse liver demonstrated a higher incidence of peroxisomes than in the control (39). Similar examination of a high dose male mouse liver did not show an increased incidence of peroxisomes. A three day study to measure liver enzyme activity following oral administration of biphenyl to male and female BDF1 mice was conducted (40). The study demonstrated that biphenyl is a weak peroxysome proliferator. It is very likely the study was conducted for too short of a time interval to show an effect. Although these authors have provided some evidence showing peroxisome proliferation can occur, it is probably insufficient to conclusively demonstrate this.

Inhalation Exposure

A 13-week vapor inhalation study using groups of 50 CD-l mice of each sex exposed to 25 or 50 ppm (160 or 320 mg/m^3; analytical concentrations) Biphenyl(37). Exposure was for 7 hours/day, 5 days/week and resulted in hyperaemia and focal hemorrhage in the lung an increase in hyperplasia of the tracheal epithelium. The effects appeared to be dose-related and partially reversible after a 30-day recovery period. In addition, the same laboratory conducted a preliminary 14-day inhalation study under essentially the same conditions and found no effects attributable to the test material (38). Both the 90-day and 14-day studies were limited in scope as only the lungs, trachea, liver, kidneys and spleen were examined microscopically. A robust summary has been prepared for the 90-day study, as it is the only subchronic study available using vapor inhalation as the exposure route. Although the study is limited in scope, it is considered useful in defining the potential of Biphenyl vapor to cause irritation of the respiratory tract.

Recommendation: No additional repeated-dose studies are recommended. The available data fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening basically involve two in vitro end-points: generally one test for mutagenicity and another for chromosomal aberrations.

Genetic Toxicology in vitro

A large number of genotoxicity studies, mostly conducted prior to 1990, have been reported on Biphenyl. The weight of evidence approach suggests that Biphenyl has little genotoxic activity. Results of the in vitro tests are shown in Table 4. Bacterial genotoxicity studies have been uniformly negative while yeast systems have suggested some degree of mutagenic and mitotic recombination activity. Testing in mammalian cells has produced mixed results with limited positive results for gene mutations and clastogenicity reported only in the presence of metabolic activation.

Closer examination has revealed a number of deficiencies in these studies. The yeast D7 mutagenicity and conversion assays (49) indicated positive activity for Biphenyl only at significantly cytotoxic levels which was two to four fold greater than currently accepted according to the guidelines. In the case of the Chinese hamster V79 gene mutation assay, test material precipitation was observed at the two highest concentrations, with the known solubility for the material known to be even lower (55). Furthermore, although no appreciable toxicity was observed during the treatment period, as determined from the number of cells harvested at the subcultivation during the expression period (>80% of control value), cloning efficiencies were not reported, hence not allowing for sufficient information for a definitive assessment. In the OECD guideline mouse lymphoma L5 178Y thymidine kinase locus assay, a weakly positive response was only observed at a single concentration that significantly inhibited cell growth [12% of control values] (57) only in the presence of S-9 following a 4-hour exposure. In a non-guideline study by the same laboratory, the proportion of single- to double-strand breaks in the same cell line was measured (63) at concentrations of 0.05-l .5 mM shown in their previous study (57) to produce significant toxicity (> 85%) when cells were allowed a 48-hour expression period. This makes the results of this alkaline unwinding assay difficult to interpret relative to the degree of cytotoxicity which would be manifest under these treatment conditions relative to prior studies in the same cell line. Thus these 4 studies were assigned reliability scores of 3 or 4.

Test System	End-point	Concentration	Result		References
			N	Y	
Salmonella typhimurium	Reverse mutations	O-5000 µg/plate	-	-	41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55
E.coli WP2, WP2 uvrA-	Gene mutations	0.1-1000 µg/ml	-	-	41, 46, 47
E. coli PQ37	DNA damage	2.4-154 µg/ml	-	-	53
Bacillus subtilis rec assav	DNA damage	no data		0	43
Saccharomyces cerevisiae D7	mutat/conversion	<154 µg/ml	+	+	49
S. cerevisiae D3	Gene conversion	no data	-	-	47, 56
Chinese hamster cells(V79)	Gene mutation	0-100 µg/ml	-	+	55
Mouse lymphoma assav	Gene mutation	0-61 µg/ml	-	(+)	57
Chinese hamster cells (CHL)	Chrom aberration	O-125 µg/ml	-	0	
Chinese hamster cells(CHL)	Chrom aberration	O-20 µg/ml	-	+	59
Chinese hamster cells (Don)	Chrom aberration	15.4-154 µg/ml		0	60
Rat hepatocytes	UDS	0.002-154 µg/ml	0	-	61, 62, 46
Chinese hamster cells(CHL)	SCE	no data		0	43
Chinese hamster cells (Don)	SCE	15.4-154 µg/ml		0	60
cells (DNA unwinding)	DNA damage	O-231 µg/ml	-	+	63
human lung fibroblasts WI38 cells)	UDS	no data	-	-	47
human tibroblasts (“nick translation assav”)	DNA damage	15.4 µg/ml	-	0	64
Y= plus S9, N = no S9, + = positive, (+) = weak positive, - = negative, 0 = no data					

Table 4. In Vitro Genotoxicity Results for ***Biphenyl***

Genetic Toxicology in vivo

Information from genotoxicity studies conducted *in vivo* is limited. In a cytogenetic assay of rat bone **marrow** cells, the incidence of chromosomal aberrations was reportedly not increased; however, details about the experimental conditions are not available (43).

Recommendation: The SIDS requirement for genetic testing has been met as assays for the evaluation of both mutagenicity and clastogenic effects have been conducted using acceptable protocols. Due to the positive results in the in vitro chromosomal aberration studies and our inability to obtain any additional information on the in vivo chromosomal aberration study, an in vivo micronucleus test will be conducted.

Reproductive Toxicity

A non-guideline multigenerational study where four successive generations of rats were exposed to dietary levels of 0, 100, 1000 or 10000 ppm Biphenyl has been conducted (66). Although this is an older study the procedure and results are reasonably well documented and it tests the reproductive toxicity of Biphenyl at increasing doses up to those that are clearly maternally and paternally toxic. Marginally reduced fertility occurred at feeding levels that were toxic to the young adult animals as manifest by reduction in weight gains prior to achieving breeding age. Feed levels that were not associated with parental toxicity did not have any effect on reproductive parameters over four generations of exposure. Biphenyl is not considered a specific reproductive toxin to the rat under these conditions. This study was conducted by a scientifically defensible method and its results are congruent with similar dosed feed studies. Because of the duration of the test over three full generations of reproduction, and the marginal effect on measured reproductive parameters, which stayed consistent over the multiple generations, this is considered an adequate test of reproductive toxicity. Additional evidence supporting a lack of reproductive toxicity is found in the 1960 chronic feeding study that incorporated two satellite reproductive and pup survival tests (34).

In addition to the available specific reproductive toxicity data, there are negative developmental toxicity studies (*vide post*). Subchronic studies also found no specific effects on reproductive organs of males or females treated with Biphenyl. For example, as part of the Japan Bioassay Research Center's subchronic study, a detailed gross and microscopic examination of male and female reproductive organs was conducted (26, 35 and 36). These studies show that even at systemically toxic doses there is no specific damage to reproductive organs of male or female experimental animals. The available reproductive data and the negative developmental and subchronic studies taken together fulfill the HPV requirement for reproductive toxicity information

Recommendation: No additional reproductive testing is recommended. The available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

Adequate developmental toxicity studies of Biphenyl have been conducted using both rats (67) and mice (68). The more recent of these studies is an EPA 1984-guideline study using four dose levels and groups of 40 mice per dose level. The results of this investigation conducted by oral gavage at 0, 125,250, 500 or 1000 mg/kg-day indicate that Biphenyl is embryotoxic at doses associated with maternal toxicity. The developmental and maternal NOAEL was found to be 500 mg/kg-day with fetotoxicity manifest as early loss. No increase in malformations was observed, even in the presence of maternal toxicity (68). The older study, published in 1979, used groups of 18-20 pregnant Wistar rats dosed by oral gavage at 0, 125,250, 500 or 1000 mg/kg-day. This study gave a result very similar with the findings in mice; Biphenyl was found to be embryotoxic at doses associated with maternal toxicity. The developmental and maternal NOAEL was found to be 500 mg/kg-day with fetotoxicity manifest as early loss. As was the case with mice, no increase in malformations was observed, even in the presence of maternal toxicity (67). Other supporting information comes from the 1960 chronic-feeding study in rats which had limited reproductive toxicity studies conducted as satellite investigations (34) and from the three-generation study that, although limited in scope, did not indicate any specific developmental toxicity. Thus, there is adequate evidence that Biphenyl is not a specific developmental toxin in rats and mice with dosing conducted by gavage and dosed feed. Taken together, the weight of evidence from these developmental toxicity studies indicates a low developmental toxicity hazard for Biphenyl.

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity with the exception of in vivo mutagenicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provides a reliable hazard assessment. In conclusion, an in vivo mutagenicity study on biphenyl will be conducted.

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